# Tofacitinib attenuates pathologic immune pathways in patients with psoriasis: A randomized phase 2 study

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**Background: Tofacitinib is an oral Janus kinase inhibitor being investigated for psoriasis.**

**Objective: We sought to elucidate the molecular mechanisms underlying the clinical efficacy of tofacitinib in patients with psoriasis.**

**Methods: Twelve patients with plaque psoriasis were randomized (3:1) to receive 10 mg of tofacitinib or placebo twice daily for 12 weeks. Biopsy specimens were taken from nonlesional (baseline) and lesional (baseline, days 1 and 3, and weeks 1, 2, 4, and 12) skin. Biopsy specimens were examined for psoriatic epidermal features (thickness, Ki67$^+$ keratinocytes and keratin 16 [KRT16] mRNA expression, and phosphorylated signal transducer and activator of transcription [pSTAT]$^+$ nuclei) and T-cell and dendritic cell (DC) subsets by using immunohistochemistry, and mRNA transcripts were quantified by using a microarray.**

**Results: In lesional skin keratinocyte pSTAT1 and pSTAT3 staining was increased at baseline but reduced after 1 day of tofacitinib (baseline, median of 1290 pSTAT1$^+$ cells/$\mu m^2$; day 1, median of 332 pSTAT1$^+$ cells/$\mu m^2$; and nonlesional, median of 155 pSTAT1$^+$ cells/$\mu m^2$). Epidermal thickness and KRT16 mRNA expression were significantly and progressively reduced after days 1 and 3 of tofacitinib administration, respectively (eg, KRT16 decreased 2.74-fold, day 3 vs baseline, $P = .016$). Decreases in DC and T-cell numbers were observed after weeks 1 and 2, respectively. At week 4, significant decreases in IL-23/$T_H17$ pathways were observed that persisted through week 12. Improvements in clinical and histologic features were strongly associated with changes in expression of psoriasis-related genes and reduction in IL-17 gene expression.**

**Conclusions: Tofacitinib has a multitiered response in patients with psoriasis: (1) rapid attenuation of keratinocyte Janus kinase/STAT signaling; (2) removal of keratinocyte-induced cytokine signaling, leading to reductions in pathologic DC and T-cell numbers to nonlesional levels; and (3) inhibition of the IL-23/$T_H17$ pathway. (J Allergy Clin Immunol 2016;137:1079-90.)**

***Key words:*** *IL-17, IL-22 family, IL-23, inflammation, Janus kinase, keratinocyte, psoriasis, phosphorylated signal transducer and activator of transcription, $T_H17$ cell, tofacitinib*

Psoriasis is a chronic immune-mediated disease characterized by epidermal hyperplasia.[1] This is driven by infiltration of T cells and dendritic cells (DCs) and associated increased cytokine levels, leading to the formation and persistence of skin plaques.[1]

Current opinion on the pathogenesis of psoriasis emphasizes the role of cytokine signaling to drive a pathogenic cycle, in which inflammatory T-cell and DC infiltrates release IL-17, IFN-γ, IL-22, and TNF, leading to the activation and proliferation of keratinocytes.[2] The stressed and dysregulated keratinocytes release chemokines, cytokines, and antimicrobial peptides (AMPs).[2] The chemokines recruit additional myeloid DCs and $T_H1$ and $T_H17$ cells, and cytokines (eg, IL-1β, IL-6, and TNF-α) enhance the proinflammatory interactions between the recruited DCs and T cells. The AMPs, including cathelicidin antimicrobial peptide (LL-37), activate pathologic DCs to produce IFN-α/β and myeloid DCs to secrete IL-12 and IL-23. These activated DCs and T cells complete the pathogenic cycle by continuing to activate keratinocytes.[2]

Tofacitinib is an oral Janus kinase (JAK) inhibitor that is being investigated for psoriasis. Tofacitinib is a small molecule with an intracellular mechanism of action against JAKs. Phase 3 studies in patients with moderate-to-severe chronic plaque psoriasis have demonstrated the efficacy of 5 and 10 mg of tofacitinib twice daily in improving clinical outcomes.[3-5]

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*Abbreviations used*
AMP: Antimicrobial peptide
DC: Dendritic cell
DEFB4A: Defensin beta 4a
FDR: False discovery rate
JAK: Janus kinase
KRT16: Keratin 16
MAD3: Meta-analysis of 3 psoriasis gene signatures
PASI: Psoriasis Area and Severity Index
pSTAT: Phosphorylated signal transducer and activator of transcription
STAT: Signal transducer and activator of transcription
TLDA: TaqMan Low Density Array
TPSS: Target Plaque Severity Score

In kinase assays tofacitinib inhibited JAK1, JAK2, and JAK3 and, to a lesser extent, tyrosine kinase 2.[6] Tofacitinib potently inhibited common γ-chain cytokines (IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21), IFN-γ, IL-6, and, to a lesser extent, IL-12 and IL-23.[6] Tofacitinib also inhibited IFN-α/β and IL-22 signaling in isolated keratinocytes *in vitro* (data on file; Pfizer Inc, New York, NY). In preclinical models tofacitinib affected both innate and adaptive immune responses and inhibited pathogenic $T_H17$ cell differentiation by inhibiting expression of IL-23 receptors.[7] However, precisely how the JAK/signal transducer and activator of transcription (STAT) pathway interacts with the multiple pathways that are central to psoriasis pathogenesis in human subjects is not completely understood.

Although improvements in psoriasis can be monitored by means of clinical assessment or histologic analysis, a more detailed understanding of therapeutic responses can be attained through the profiling of disease-related and inflammatory mRNA. Previous studies have established that cytokine-targeted biologics (eg, TNF-α, IL-17, or IL-23 inhibitors) are efficacious for the treatment of psoriasis and have the ability to inhibit a central IL-23/$T_H17$ axis of psoriasis, with strong downregulation of a series of genes that are induced in keratinocytes and other cells by these proinflammatory cytokines.[8-12] Suppression of "core" disease-defining genes as a result of treatment can be demonstrated by gene profiling, which allows the relative suppression of different proinflammatory pathways to be quantitatively compared.[13-15] This molecular analysis allows residual expression of disease-related genes to be detected and quantified in lesions that have resolved clinically and histologically.

This phase 2 study aimed to elucidate the cellular and molecular mechanisms through which tofacitinib improves clinical manifestations of psoriasis by delineating the time course of changes in the pathogenic cycle of psoriasis.

# METHODS

## Patients

Eligible patients were 18 years or older with a diagnosis of moderate-to-severe plaque-type psoriasis for 12 or more months. Patients with a recent infection, current malignancy, or history of malignancy (except adequately treated or excised basal/squamous cell carcinoma or cervical carcinoma *in situ*) or evidence of active or latent tuberculosis infection were excluded.

## Study design

This was a phase 2, randomized, placebo-controlled, double-blind study carried out in 6 centers in the United States from March 2013 to November 2013 (clinicaltrials.gov: NCT01710046). Patients were randomized 3:1 to receive 10 mg of oral tofacitinib or placebo twice daily for 12 weeks by using an automated Web or telephone randomization system.

At each study visit (baseline, days 1 and 3, and weeks 1, 2, 4, and 12), punch biopsy specimens were collected from each patient before the morning study drug dose (full details are provided in the Methods section in this article's Online Repository at www.jacionline.org). Baseline biopsy specimens were taken from lesional and nonlesional skin; at all later study visits, biopsy specimens were only taken from lesional skin. Clinical assessments at baseline and weeks 1, 2, 4, and 12 included the Psoriasis Area and Severity Index (PASI; overall measure of disease severity) and Target Plaque Severity Score (TPSS; individual lesion assessment of severity of erythema, induration, and scaling).

## Immunohistochemistry and quantitative cell counting

Procedures for immunohistochemistry and quantitative cell counting are outlined in greater detail in the Methods section and Table E4 in this article's Online Repository. Biopsy specimens from lesional and nonlesional skin were stained for phosphorylated signal transducer and activator of transcription (pSTAT) 1 and pSTAT3, and numbers of epidermal and dermal reactive nuclei per square millimeter were quantified by using Definiens Tissue Studio software (Definiens AG, München, Germany). Skin biopsy specimens were also evaluated for expression of keratin 16 (KRT16); Ki67 (MKI67); cluster of differentiation antigens CD3, CD11c, and CD8; langerin (CD207); lysosomal-associated membrane protein 3 (LAMP3); S100 calcium-binding protein A7 (S100A7); MX dynamin-like GTPase 1/2 (MX1/MX2); human β-defensin (HBD2, defensin beta 4A [DEFB4A]); and S100A8/9 (S100A8), and the number of positive cells per square millimeter of epidermis was counted manually by using National Institutes of Health Image 6.1 software (http://rsb.info.nih.gov/nih-image).

## Quantitative RT-PCR and microarray analyses

RNA was extracted from biopsy specimens by using the RNeasy Mini Kit (Qiagen, Valencia, Calif). The quality of extracted RNA was examined by using the Agilent Bioanalyzer 2100 (Agilent Technologies, Santa Clara, Calif). IL-23p40 (IL-12B) quantitative RT-PCR was performed with EZ PCR core reagents, primers, and probes (Life Technologies, Grand Island, NY), as previously published,[16] and the result was normalized to the ribosomal protein, large P0 (RPLP0) housekeeping gene. For microarray analysis, RNA was amplified, labeled, and hybridized by using a standard protocol (Ovation Whole Blood Solution and Encore Biotin Module; NuGEN Technologies, San Carlos, Calif) to GeneChip Human Genome U133A 2.0 Arrays (Affymetrix, Santa Clara, Calif) to measure relative gene expression. The data discussed in this publication have been deposited in the National Center for Biotechnology Information's Gene Expression Omnibus and are accessible through GEO Series accession number GSE69967.

## TaqMan Low Density Array analysis

Samples for quantitative RT-PCR analysis using the Custom TaqMan Array Card (Life Technologies) were processed by Asuragen (Austin, Tex), according to the company's standard operating procedures (full details are provided in the Methods section and Fig E4 in this article's Online Repository at www.jacionline.org). A total of 192 inflammation/cytokine-related genes were analyzed, including 185 targets, 5 normalization genes, and 2 plate controls.

## Statistical analysis

The study sample size was based on the objective of exploring the relationship between efficacy end points and exploratory gene markers and was not specifically powered for reaching statistical significance.

A randomization ratio of 3:1 was used to enroll approximately 9 patients in the tofacitinib group and 3 patients in the placebo group. The sample size of 9 for the tofacitinib group provided an 81% probability of observing a mean reduction in gene expression level of at least 70% among responders, whereas a sample size of 3 for the placebo group would ensure less than 1% probability to observe a mean reduction of 70% or more in the placebo group (further details are provided in the Methods section in this article's Online Repository). Statistical analysis was carried out with R-language[17] and packages available through the Bioconductor project.[18] Tofacitinib-treated patients were classified as responders or nonresponders based on expression of the KRT16 gene in target lesions (patients with a decrease of 75% of the difference between baseline lesional and nonlesional skin were considered responders). Longitudinal profiles of clinical end points and gene expression were compared between the tofacitinib and placebo groups and between responders and nonresponders by using mixed-effect models with time and treatment/response as fixed factors and patient as a random effect. Comparisons of clinical data and immunohistochemistry counts are presented with *P* values (significance at $P = .05$). Comparisons of high-throughput data (microarray and TaqMan Low Density Array [TLDA]) were corrected for multiple hypothesis testing and are presented with false discovery rate (FDR) values (significance level = 0.1). Further details of the data handling procedures and statistical analysis of TLDA and microarray data can be found in the Methods section in this article's Online Repository. Data collection and analyses were carried out by authors from both Pfizer Inc and Rockefeller University, and therefore the article includes data that Pfizer Inc is not responsible for validating/storing.

## RESULTS

### Patients

Twelve patients were randomized to 10 mg of tofacitinib twice daily (n = 9) or placebo (n = 3, see Fig E1 in this article's Online Repository at www.jacionline.org). One patient treated with tofacitinib treatment was lost to follow-up after week 4; data were included in analyses up to this time point. Eleven patients completed the 12-week study. Demographics and baseline characteristics are reported in Table E1 in this article's Online Repository at www.jacionline.org.

### Clinical and histologic markers of psoriasis

At baseline, mean PASI scores were 21.9 and 23.3 for tofacitinib- and placebo-treated patients, respectively (Fig 1, *A*), and histologic features of psoriasis were significantly increased in lesional versus nonlesional skin in both treatment groups (Fig 1, *B-D*). PASI scores decreased from baseline through week 12 in tofacitinib-treated patients (Fig 1, *A*). Corresponding improvements in histologic features of psoriasis are shown in Fig 1, *B* to *D*. Response to tofacitinib was rapid, with significant reductions in epidermal thickness and Ki67 levels from baseline after 1 day of treatment ($P < .05$) and significant reductions in KRT16 expression after 3 days of treatment ($P < .05$). All 3 measures showed progressively greater reductions from baseline thereafter. Changes in clinical and histologic measures with placebo were generally numerically lower and highly variable over time. The number of responders (≥75% reduction in KRT16 expression from baseline patients with a decrease of 75% of the difference between baseline lesional and nonlesional skin were considered responders) was 6 (75%) with tofacitinib and 1 (33%) with placebo; the reason for response in the placebo-treated patient is unknown.

Representative histologic assessments performed on biopsy specimens from a tofacitinib-treated responder demonstrated a decrease in psoriasiform pattern and overall epidermal thickness over time (Fig 2). After 3 days of treatment, KRT16 expression was only focally present, and by week 2, the epidermis no longer showed the presence of KRT16 in suprabasal keratinocytes. After 4 weeks, KRT16 staining was virtually nonexistent in lesional skin, and by week 12, the overall phenotype of tofacitinib-treated lesional skin was virtually identical to that of baseline nonlesional skin.

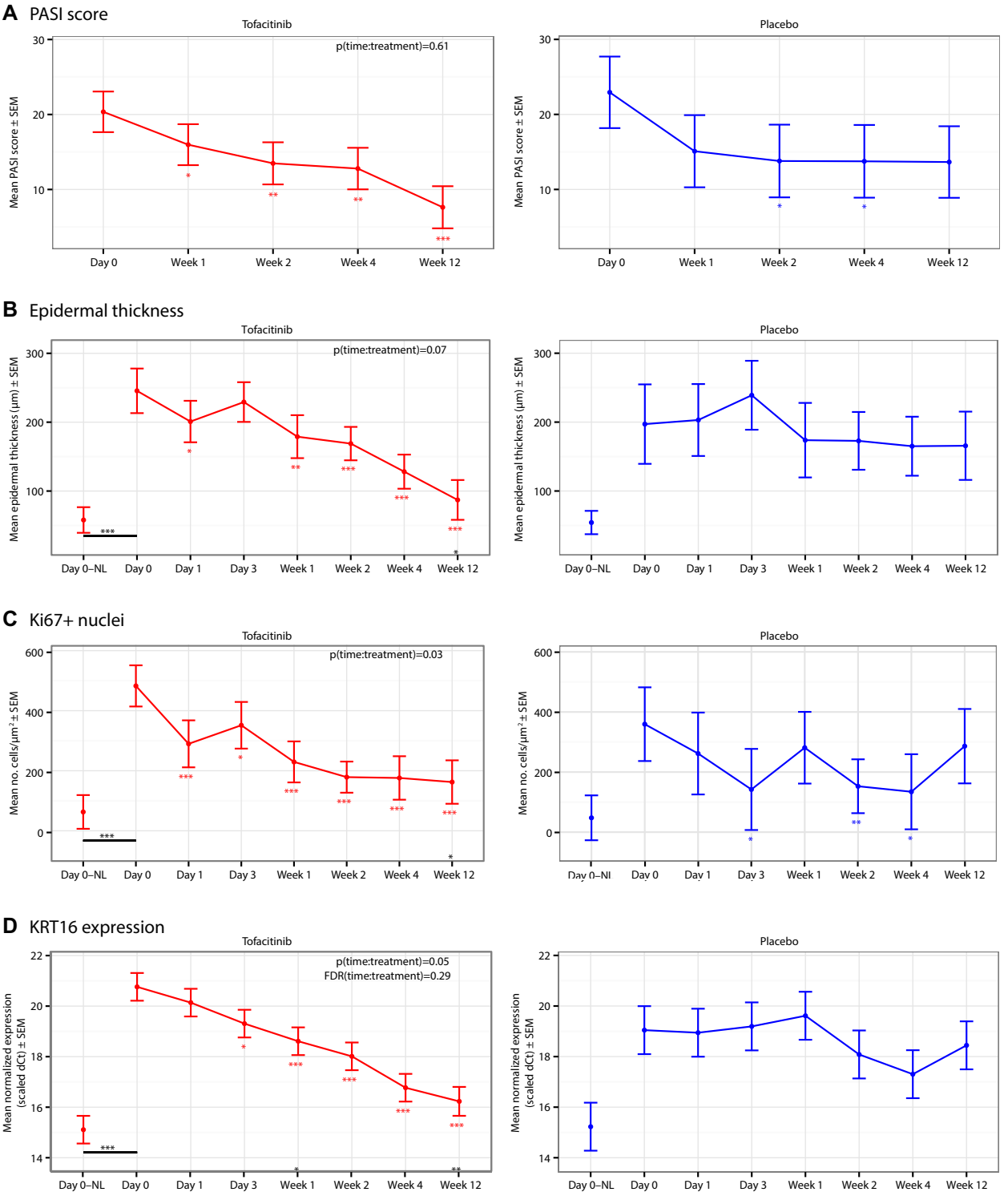
### JAK/STAT pathway inhibition

pSTAT expression was measured to assess the inhibition of JAK/STAT signaling pathways by tofacitinib. Numbers of baseline $pSTAT1^+$ and $pSTAT3^+$ nuclei were greater in lesional skin relative to nonlesional skin (lesional = median of 1290 $pSTAT1^+$ cells/$\mu m^2$ of epidermis and median of 281 $pSTAT3^+$ cells/$\mu m^2$ of epidermis; nonlesional = median of 155 $pSTAT1^+$ cells/$\mu m^2$ of epidermis and median of 16.5 $pSTAT3^+$ cells/$\mu m^2$ of epidermis; Fig 3). Rapid decreases in numbers of $pSTAT1^+$ and $pSTAT3^+$ nuclei in epidermal keratinocytes were observed after 1 day of tofacitinib treatment (median of 332 $pSTAT1^+$ cells/$\mu m^2$ of epidermis and median of 23 $pSTAT3^+$ cells/$\mu m^2$ of epidermis, Fig 3), indicating a rapid and direct effect on keratinocyte signaling. Placebo-treated patients continued to express pSTAT1 and pSTAT3 at baseline levels in lesional skin throughout the study (Fig 3).

### Psoriasis-related gene expression

Gene expression profiling was performed on Affymetrix GeneChips to compare the expression of psoriasis-related genes in the psoriasis transcriptome over time between tofacitinib- and placebo-treated patients (Fig 4, *A*). Genes that were highly upregulated in lesional skin at baseline showed a rapid decrease over the first 2 weeks of tofacitinib treatment, and by week 12, expression levels were virtually identical to those of nonlesional skin (Fig 4, *A* and *C*). Normalization of gene expression to nonlesional levels was not seen in placebo-treated patients. The full set of genes that were modulated by tofacitinib is listed in Table E2 in this article's Online Repository at www.jacionline.org.

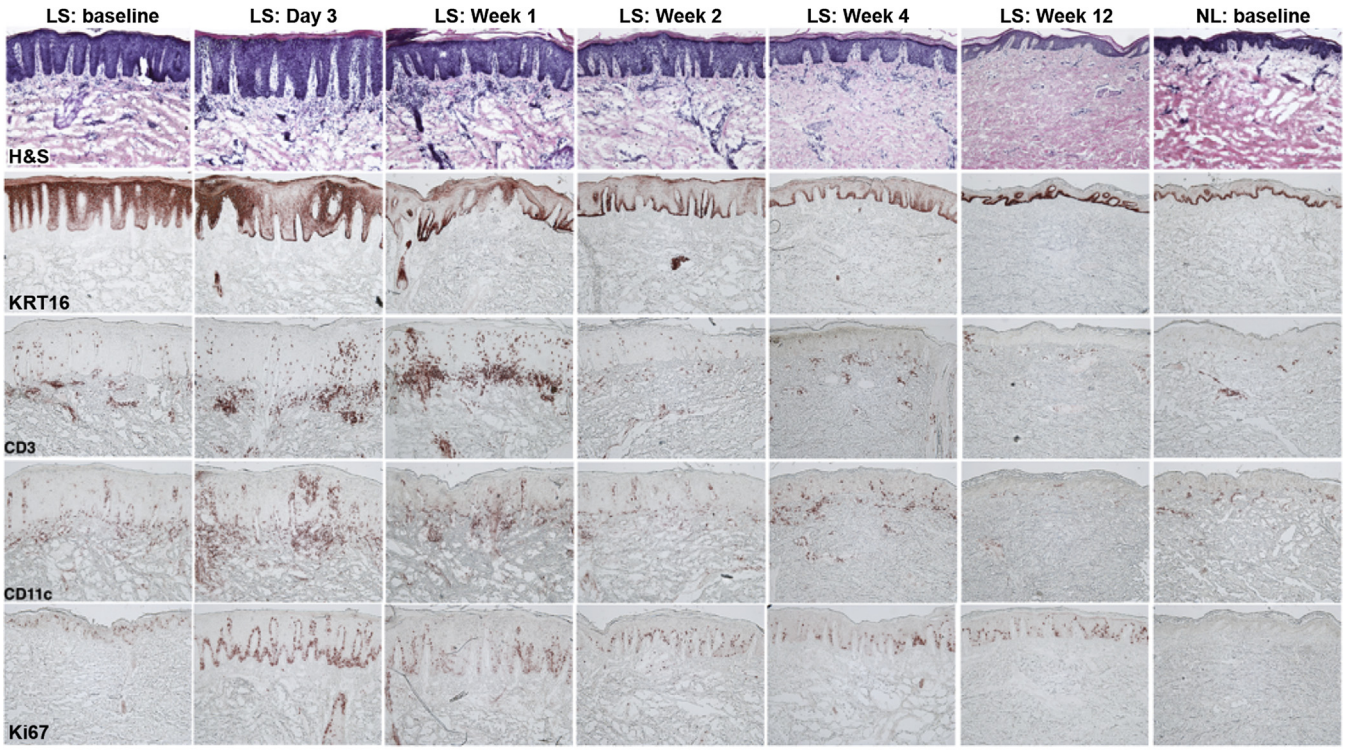
Cytokine gene expression was measured by using quantitative RT-PCR to confirm the gene array results. Fig 5 shows the expression of selected cytokine transcripts, along with corresponding changes in $CD11c^+$ DC and $CD3^+$ T-cell numbers. Reductions in these cell levels appeared gradually over time, with significant decreases in $CD11c^+$ DC and $CD3^+$ T-cell numbers after 1 and 2 weeks of tofacitinib treatment (FDR < 0.1 and FDR < 0.05, respectively; Fig 5, *A* and *B*). At week 12, DC and T-cell numbers in tofacitinib-treated patients in lesional skin were similar to those observed in nonlesional skin and were significantly reduced versus those in placebo-treated patients (both FDR < 0.1). Expression of IL-12B, the cytokine p40 subunit common to IL-23 and IL-12, which is released by DCs and activates $T_H17$ and $T_H1$ cells, was significantly reduced after 2 weeks (FDR < 0.05; Fig 5, *C*). Reduction in expression of the T-cell–related cytokines IFN-γ and IL-17 occurred relatively late, with significant reductions observed after 4 weeks of treatment (FDR < 0.01 and FDR < 0.001, respectively; Fig 5, *D* and *E*) after the onset of improvements in clinical and histologic features of psoriasis. Expression of the IL-17–regulated molecules IL-19 and CCL20 were significantly reduced after 2 and 4 weeks (FDR < 0.05 and FDR < 0.01, respectively; Fig 5, *F* and *H*). IL-22 expression




*P < .05, **P < .01, ***P < .001

Data presented as mean ± SEM; * or * indicates significance versus baseline;
* above the x-axis in the tofacitinib plots indicates significant difference in change from baseline with tofacitinib versus placebo;
* in the tofacitinib plots indicates significance of lesional versus nonlesional skin for all subjects (tofacitinib and placebo) at baseline.
P(time:treatment): P value of the F-test associated to the time-by-treatment interaction term in the mixed model.
FDR(Time:treatment): adjustment for multiplicity for P(time:treatment) using the Benjamini-Hochberg approach.
dCt, delta cycle threshold; FDR, false discovery rate; KRT16, keratin 16; NL, nonlesional skin; PASI, Psoriasis Area and Severity Index;
SEM, standard error of the mean.

**FIG 1.** Clinical and histologic features of psoriasis over time with tofacitinib and placebo in lesional skin (except where indicated). **A,** PASI score. **B,** Epidermal thickness. **C,** Ki67$^{+}$ nuclei. **D,** KRT16 expression.




Images taken at 100x magnification.
CD, cluster of differentiation; DC, dendritic cell; H&S, hematoxylin and eosin; KRT16, keratin 16; LS, lesional skin; NL, nonlesional skin.

**FIG 2.** Histologic markers of psoriasis in a tofacitinib-treated patient. Hematoxylin and eosin *(H&S)* stain shows a thickened epidermis with elongation into the dermis in lesional skin. KRT16 staining shows increased keratinocyte proliferation in the epidermis, and CD3 and CD11c staining shows accumulation of lymphocytes and DCs, respectively, in lesional versus nonlesional skin. Ki67 staining shows cell proliferation in the epidermis.

was significantly reduced after 1 week of tofacitinib treatment (FDR < 0.1; Fig 5, *G*).

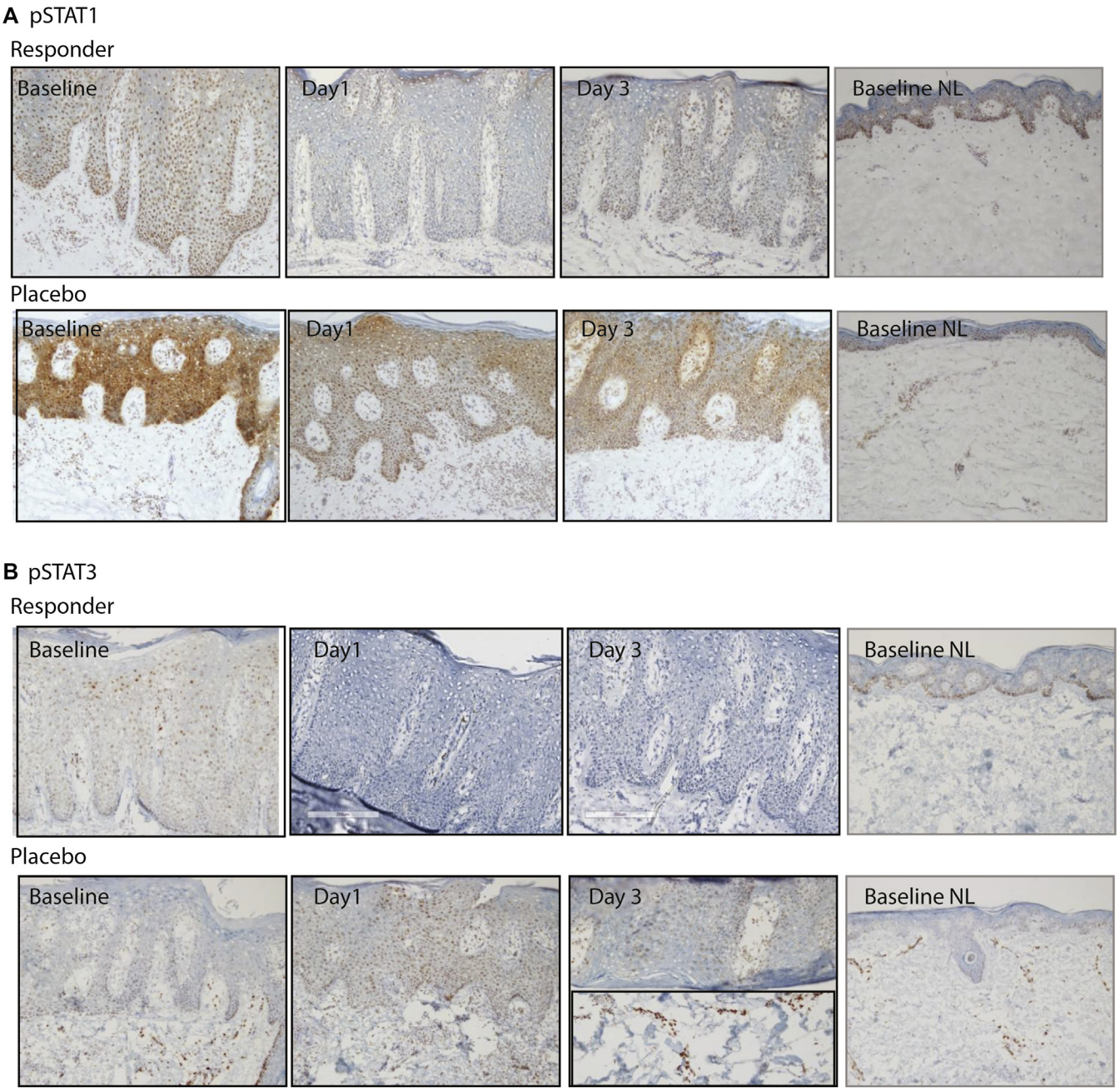
In light of the rapid reduction in nuclear staining for both pSTAT and Ki67 in keratinocytes, which suggested a direct effect of tofacitinib on keratinocytes, those genes that showed differential expression at early time points were more closely examined (Fig 4, *B*, and Table I). MX dynamin-like GTPase 2 interferon response gene (MX2), interferon-induced protein with tetratricopeptide repeats 1 (IFIT1), and ubiquitin-specific peptidase 18 (USP18) expression was decreased in the first day of dosing (Fig 4, *B*), which is consistent with the direct role of JAKs in IFN-α/β signaling. Expression of keratinocyte proliferation markers, including the S100 proteins, lipocalin-2 (LCN2), defensin beta 4A (DEFB4A), chemokine (C-X-C motif) ligand 1 (CXCL1), amphiregulin (AREG), and kallikrein-related peptidase (KLK) 6 and 10, was examined, and DEFB4A, CXCL1, AREG, and KLK10 expression decreased by week 2 (Table I) before reduction in IL-17 expression. Expression of IL-19, IL-20, and IL-24, which are produced by IL-17– and IL-22–stimulated keratinocytes and signal in a JAK-dependent manner,[19] was also reduced by week 2, before reduction of IL-17 expression (Table I). Expression of IL-36α (IL36A) and IL-36γ (IL36G), which are induced by IL-17 and IL-22 and can drive keratinocyte proliferation and enhance production of defensins and antimicrobial products,[20] also decreased by week 2 (IL-36γ, TLDA only by week 2; Table I).

IL-37, an anti-inflammatory member of the IL-1 family that competes with IL-18 to decrease expression of proinflammatory molecules by keratinocytes,[21] was expressed at low levels in lesional skin but rapidly increased with treatment (Table I).

## Association between clinical and histologic improvement and gene expression changes

Although PASI scores showed significant improvement after 2 weeks of tofacitinib treatment ($P < .001$, see Fig E2, *A*, in this article's Online Repository at www.jacionline.org), significant improvement from baseline in plaque characteristics (TPSS) were observed as early as week 1 ($P < .05$, see Fig E2, *B*). Placebo-treated patients also showed a significant decrease in PASI scores from baseline, but changes in TPSSs were not significant. Psoriasis transcriptome gene expression (see Fig E2, *C* and *D*) showed a gradual decrease with tofacitinib, with significant reductions from baseline versus placebo observed at week 1 (FDR < 0.1). Similar gene expression profiles were reported by using TLDA and microarray analyses. Additional analyses of changes in gene expression with tofacitinib treatment using superenhancers of $T_H17$, $T_H1$, and $T_H2$ cells are presented in Fig E3 in this article's Online Repository at www.jacionline.org, with further details in the accompanying text.

A correlation analysis was carried out to assess whether changes in expression of psoriasis transcriptome genes and cytokine expression were related to improved clinical and histologic features of psoriasis with tofacitinib (Fig 6, *A*). The psoriasis transcriptome meta-analysis of 3 psoriasis gene signatures (MAD3)

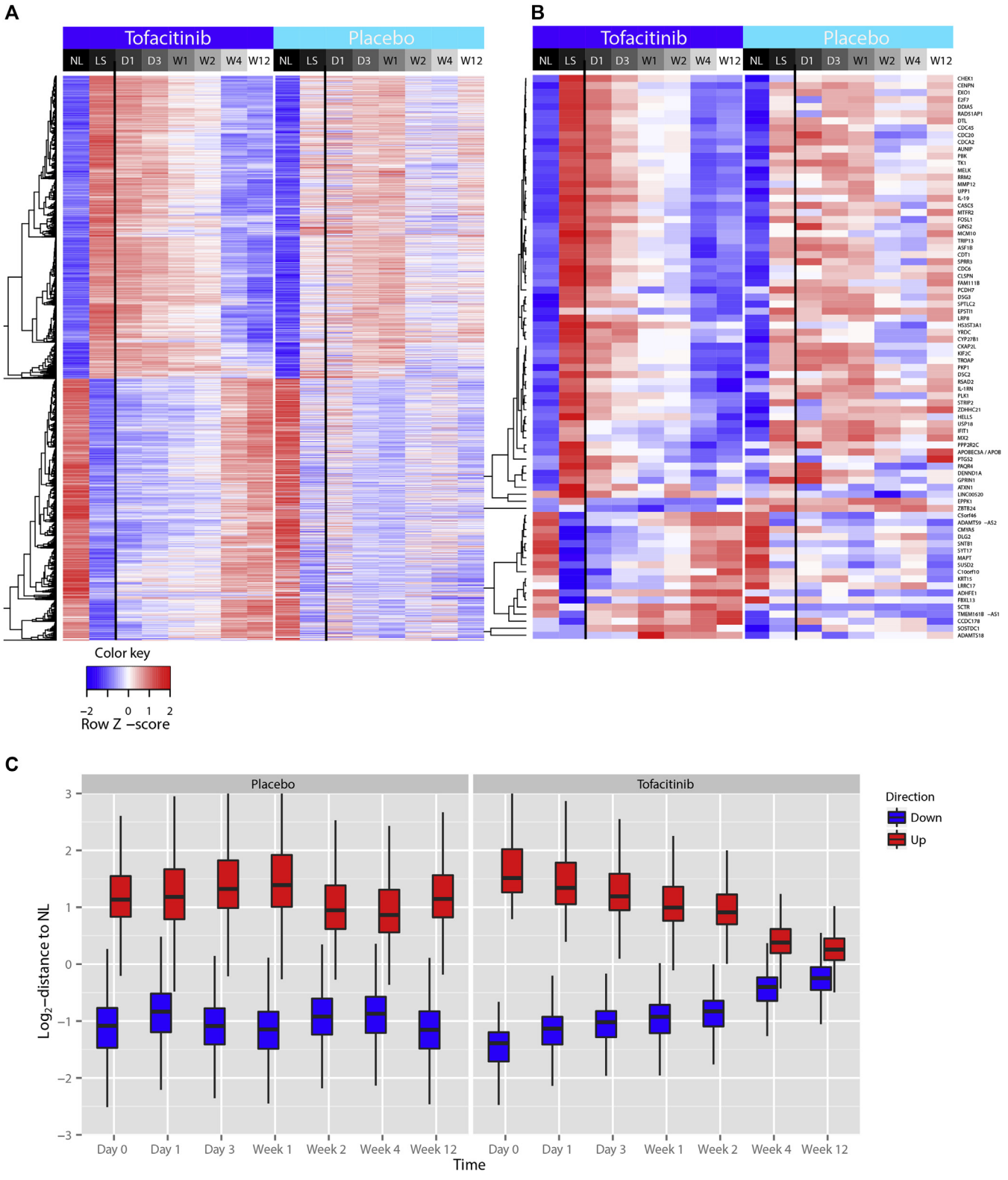



Images taken at 100x magnification.
NL, nonlesional skin; pSTAT, phosphorylated signal transducer and activator of transcription.

**FIG 3.** pSTAT1 and pSTAT3 expression in lesional biopsy specimens (except where indicated) from a tofacitinib-treated responder and a placebo-treated nonresponder. **A,** pSTAT1 expression. **B,** pSTAT3 expression.

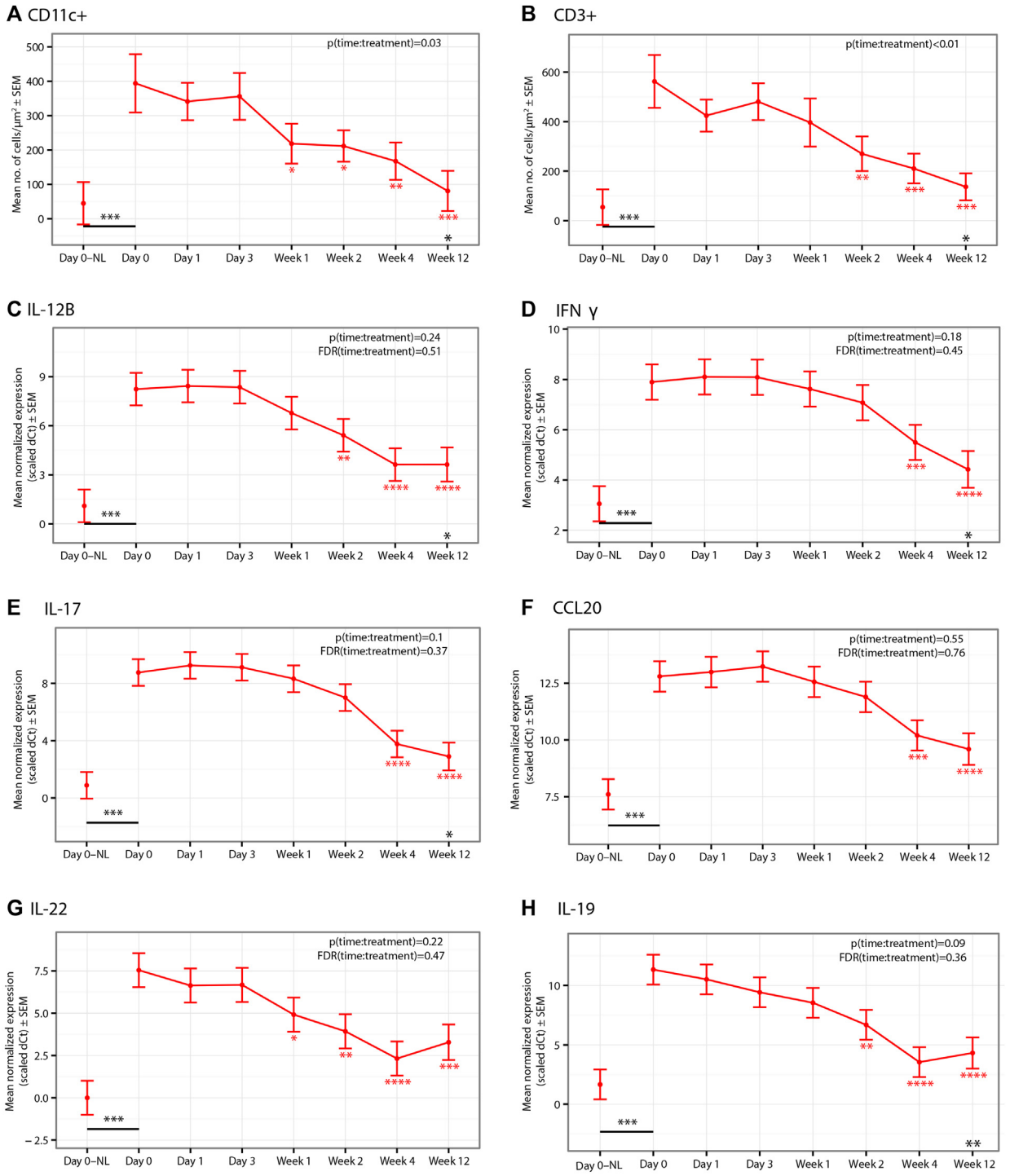
score and IL-17 expression were both highly correlated with KRT16 expression, and the MAD3 score was highly correlated with epidermal thickness. IL-17 gene expression and MAD3 scores were both negatively correlated with PASI score improvement. Gene expression measures showed stronger correlation with histologic measures of disease activity (KRT16 and epidermal thickness) than with clinical assessment (PASI score).

The relationship between changes in the psoriasis transcriptome and IL-17 expression and the histologic response to tofacitinib was further verified by comparison of psoriasis gene expression between tofacitinib responders and nonresponders (Fig 6, *B*). Responders showed downregulation of gene expression in lesional skin after 1 week of treatment, coinciding with clinical improvements; gene expression levels approached those of nonlesional skin after 4 to 12 weeks. In general, no change in gene expression was observed in nonresponders over the course of the study. The full set of genes that were differentially expressed between responders and nonresponders is listed in Table E3 in this article's Online Repository at www.jacionline.org.




D, day; FCH, fold change; FDR, false discovery rate; LS, lesional skin; NL, nonlesional skin; W, week.

**FIG 4.** Expression of psoriasis transcriptome genes. **A,** Heat map showing mean expression profiles for genes in the psoriasis transcriptome with tofacitinib and placebo. **B,** Early effects of tofacitinib: heat map showing genes significantly changed after 1 week of treatment (FDR < 0.05; fold change > 2). **C,** Comparison with nonlesional skin for genes that were upregulated and downregulated in the psoriasis transcriptome in lesional skin.




*FDR<0.1, **FDR<0.05, ***FDR<0.01, ****FDR<0.001

Data presented as mean ± SEM; * indicates significance versus baseline;
* above the x-axis in the tofacitinib plots indicates significant difference in change from baseline with tofacitinib versus placebo;
* in the tofacitinib plots indicates significance of lesional versus nonlesional skin for all subjects (tofacitinib and placebo) at baseline.
P(time:treatment): P value of the F-test associated to the time-by-treatment interaction term in the mixed model.
FDR(Time:treatment): adjustment for multiplicity for P(time:treatment) using the Benjamini-Hochberg approach.
CCL20, chemokine (C-C motif) ligand 20; CD, cluster of differentiation; dCt, delta cycle threshold; FDR, false discovery rate;
IFNγ, interferon-γ; IL, interleukin; NL, nonlesional skin; SEM, standard error of the mean.

**FIG 5.** Inflammatory cell and cytokine expression with tofacitinib in lesional skin (except where indicated). **A,** DCs ($CD11c^+$ cells). **B,** T cells ($CD3^+$ cells). **C,** IL-12B. **D,** IFN-γ. **E,** IL-17. **F,** CCL20. **G,** IL-22. **H,** IL-19.

**TABLE I.** $Log_2$-fold changes from baseline in gene expression of selected keratinocytes proliferation markers induced by tofacitinib

| $Log_2$-fold change (SEM) | Day 1 | Day 3 | Week 1 | Week 2 | Week 4 | Week 12 |
|---|---|---|---|---|---|---|
| Microarray | | | | | | |
| IL-19 | −1.22 (0.85) | −1.53 (0.85) | −2.87 (0.85)† | −3.71 (0.85)‡ | −4.78 (0.85)§ | −4.23 (0.88)§ |
| IL-20 | −0.33 (0.40) | −0.58 (0.40) | −1.01 (0.40) | −1.32 (0.40)† | −1.89 (0.40)§ | −1.97 (0.41)§ |
| IL-22 | −0.02 (0.38) | −0.29 (0.38) | −1.01 (0.38) | −0.99 (0.38)* | −1.49 (0.38)‡ | −1.35 (0.40)‡ |
| IL-24 | −0.74 (0.39) | −0.74 (0.39) | −0.98 (0.39) | −1.56 (0.39)† | −2.01 (0.39)§ | −1.72 (0.40)§ |
| IGF1R | 0.25 (0.23) | 0.36 (0.23) | 0.54 (0.23) | 0.64 (0.23)* | 1.02 (0.23)§ | 0.98 (0.24)§ |
| TNF | 0.17 (0.26) | 0.63 (0.26) | 0.07 (0.26) | −0.10 (0.26) | −0.30 (0.26) | −0.65 (0.27)† |
| AREG | −0.10 (0.35) | −0.64 (0.35) | −1.02 (0.35)* | −1.38 (0.35)† | −2.32 (0.35)§ | −2.35 (0.36)§ |
| IL-6 | −0.39 (0.44) | −0.46 (0.44) | −0.79 (0.44) | −1.05 (0.44) | −1.27 (0.44)† | −0.90 (0.45)* |
| IL-36A | −0.80 (0.79) | −1.84 (0.79) | −1.98 (0.79) | −2.78 (0.79)† | −4.13 (0.79)§ | −4.38 (0.82)§ |
| IL-36G | −0.13 (0.46) | −0.40 (0.46) | −0.70 (0.46) | −1.09 (0.46) | −2.58 (0.46)§ | −3.08 (0.48)§ |
| IL-37 | 0.65 (0.49) | 0.56 (0.49) | 1.16 (0.49) | 1.53 (0.49)† | 2.15 (0.49)§ | 2.25 (0.50)§ |
| IL-1B | 0.09 (0.58) | −0.21 (0.58) | −1.26 (0.58) | −1.58 (0.58)* | −2.99 (0.58)§ | −2.81 (0.60)§ |
| S100A8 | −0.06 (0.40) | −0.03 (0.40) | −0.16 (0.40) | −0.40 (0.40) | −1.12 (0.40)† | −1.98 (0.41)§ |
| S100A9 | −0.19 (0.77) | −0.31 (0.77) | −0.70 (0.77) | −1.24 (0.77) | −3.17 (0.77)§ | −4.58 (0.79)§ |
| S100A12 | −0.08 (0.96) | −0.43 (0.96) | −1.01 (0.96) | −2.25 (0.96) | −5.55 (0.96)§ | −5.86 (1.00)§ |
| LCN2 | −0.01 (0.81) | −0.25 (0.81) | −0.63 (0.81) | −1.24 (0.81) | −3.04 (0.81)‡ | −4.22 (0.84)§ |
| DEFB4A | −0.02 (1.05) | −0.14 (1.05) | −0.46 (1.05) | −2.00 (1.05) | −4.47 (1.05)§ | −5.82 (1.09)§ |
| CXCL1 | −0.58 (0.77) | −1.06 (0.77) | −2.10 (0.77)* | −3.10 (0.77)† | −4.55 (0.77)§ | −4.26 (0.80)§ |
| KLK6 | −0.25 (0.89) | −0.97 (0.89) | −1.30 (0.89) | −1.79 (0.89) | −3.34 (0.89)‡ | −3.74 (0.92)§ |
| KLK10 | −0.52 (0.33) | −0.53 (0.33) | −0.71 (0.33) | −0.92 (0.33)* | −1.82 (0.33)§ | −2.46 (0.34)§ |
| TLDA | | | | | | |
| IL-19 | −0.82 (1.51) | −1.92 (1.51) | −2.80 (1.51) | −4.65 (1.51)† | −7.79 (1.51)§ | −6.95 (1.51)§ |
| IL-20 | −0.12 (1.23) | −0.70 (1.23) | −2.19 (1.23) | −2.92 (1.23)† | −5.22 (1.23)§ | −5.12 (1.23)§ |
| IL-22 | −0.91 (1.22) | −0.87 (1.22) | −2.63 (1.22) | −3.61 (1.22)† | −5.22 (1.22)§ | −4.22 (1.22)‡ |
| IL-24 | −1.63 (1.17) | −2.13 (1.17) | −2.27 (1.17) | −3.59 (1.17)† | −5.44 (1.17)§ | −5.36 (1.17)§ |
| IGF1R | – | – | – | – | – | – |
| TNF | 0.02 (0.26) | 0.36 (0.26) | −0.02 (0.26) | −0.01 (0.26) | −0.36 (0.26)* | −0.37 (0.26)* |
| AREG | 0.11 (0.30) | −0.20 (0.30) | −0.54 (0.30) | −0.80 (0.30)† | −1.70 (0.30)§ | −1.81 (0.30)§ |
| IL-6 | 0.14 (0.55) | 0.47 (0.55) | 0.12 (0.55) | −0.30 (0.55) | −0.53 (0.55) | −0.44 (0.55) |
| IL-36A | −0.18 (1.48) | −1.09 (1.48) | −1.75 (1.48) | −3.96 (1.48)† | −7.96 (1.48)§ | −8.30 (1.48)§ |
| IL-36G | 0.00 (0.55) | −0.25 (0.55) | −0.77 (0.55) | −1.33 (0.55)† | −3.06 (0.55)§ | −3.54 (0.55)§ |
| IL-37 | 0.75 (0.43) | 0.85 (0.43) | 1.14 (0.43)† | 1.42 (0.43)‡ | 2.00 (0.43)§ | 2.16 (0.43)§ |
| IL-1B | 0.00 (0.61) | −0.07 (0.61) | −1.26 (0.61)* | −1.26 (0.61)* | −2.32 (0.61)§ | −2.38 (0.61)§ |
| S100A8 | −0.08 (1.00) | −0.48 (1.00) | −1.09 (1.00) | −2.13 (1.00)* | −4.86 (1.00)§ | −6.24 (1.00)§ |
| S100A9 | −0.06 (0.97) | −0.54 (0.97) | −1.06 (0.97) | −2.04 (0.97)* | −4.77 (0.97)§ | −6.25 (0.97)§ |
| S100A12 | 0.08 (1.01) | −0.36 (1.01) | −0.96 (1.01) | −2.00 (1.01)* | −5.22 (1.01)§ | −5.80 (1.01)§ |
| LCN2 | 0.23 (0.83) | −0.08 (0.83) | −0.83 (0.83) | −1.52 (0.83)* | −3.48 (0.83)§ | −4.20 (0.83)§ |
| DEFB4A | −0.12 (1.50) | −0.85 (1.50) | −1.72 (1.50) | −3.78 (1.50)† | −7.49 (1.50)§ | −9.27 (1.50)§ |
| CXCL1 | −0.55 (0.66) | −0.44 (0.66) | −1.56 (0.66)* | −2.12 (0.66)† | −3.48 (0.66)§ | −2.95 (0.66)§ |
| KLK6 | −0.12 (0.79) | −0.86 (0.79) | −1.22 (0.79) | −1.78 (0.79)† | −3.03 (0.79)§ | −3.43 (0.79)§ |
| KLK10 | −0.29 (0.37) | −0.46 (0.37) | −0.72 (0.37) | −0.95 (0.37)† | −1.96 (0.37)§ | −2.53 (0.37)§ |

*AREG*, Amphiregulin; *CXCL*, chemokine (C-X-C motif) ligand; *IGF1R,* insulin-like growth factor 1 receptor; *KLK*, kallikrein-related peptidase; *LCN*, lipocalin; *S100*, s100 calcium-binding protein; *TNF*, tumor necrosis factor.
*FDR < 0.1.
†FDR < 0.05.
‡FDR < 0.001.
§FDR < 0.0001.

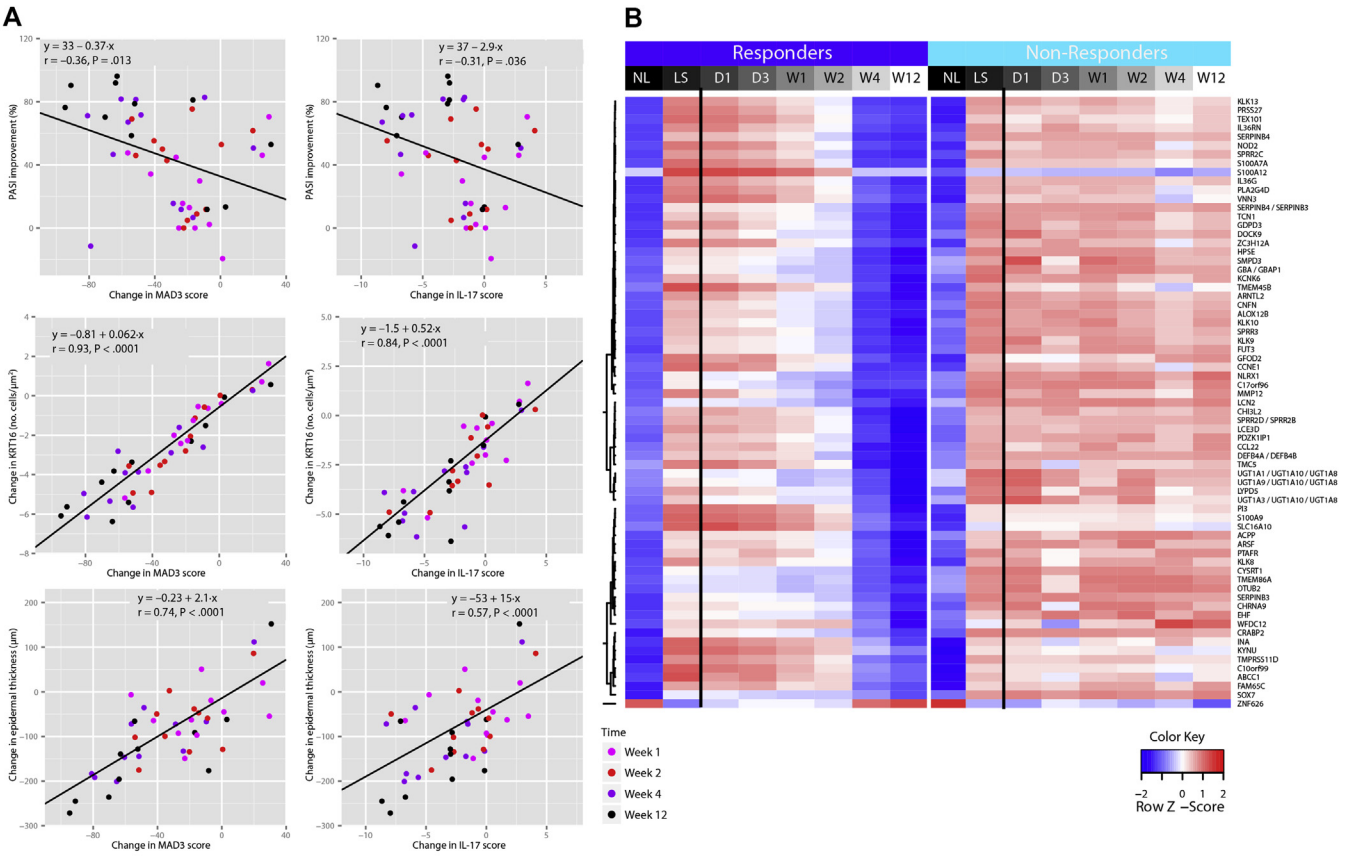
## Safety

Four patients (n = 2 per group) experienced a total of 8 adverse events. Most adverse events were mild in severity (6 mild, 1 moderate, and 1 severe). One case of cholelithiasis in a placebo-treated patient was classified as a serious adverse event, resulting in temporary discontinuation of study medication.

## DISCUSSION

Psoriasis is characterized by a pathogenic cycle in which inflammatory T-cell and DC infiltrates within the skin release cytokines, leading to the activation and proliferation of keratinocytes.[2] These activated keratinocytes release chemokines, which in turn recruit and activate more myeloid DCs and $T_H1$ and $T_H17$ cells to continue the pathogenic cycle.[2] The results presented here suggest that tofacitinib has a unique multitiered mechanism of action with an early direct effect on keratinocytes. This mechanism manifests itself histologically through a rapid decrease in markers of keratinocyte proliferation and epidermal thickness and a normalization of inflammatory infiltrate, eventually leading to a decrease in the IL-23/$T_H17$ axis that drives keratinocyte dysregulation (Fig 7).

We have provided the following evidence for a direct effect of tofacitinib on keratinocytes. At baseline, levels of nuclear

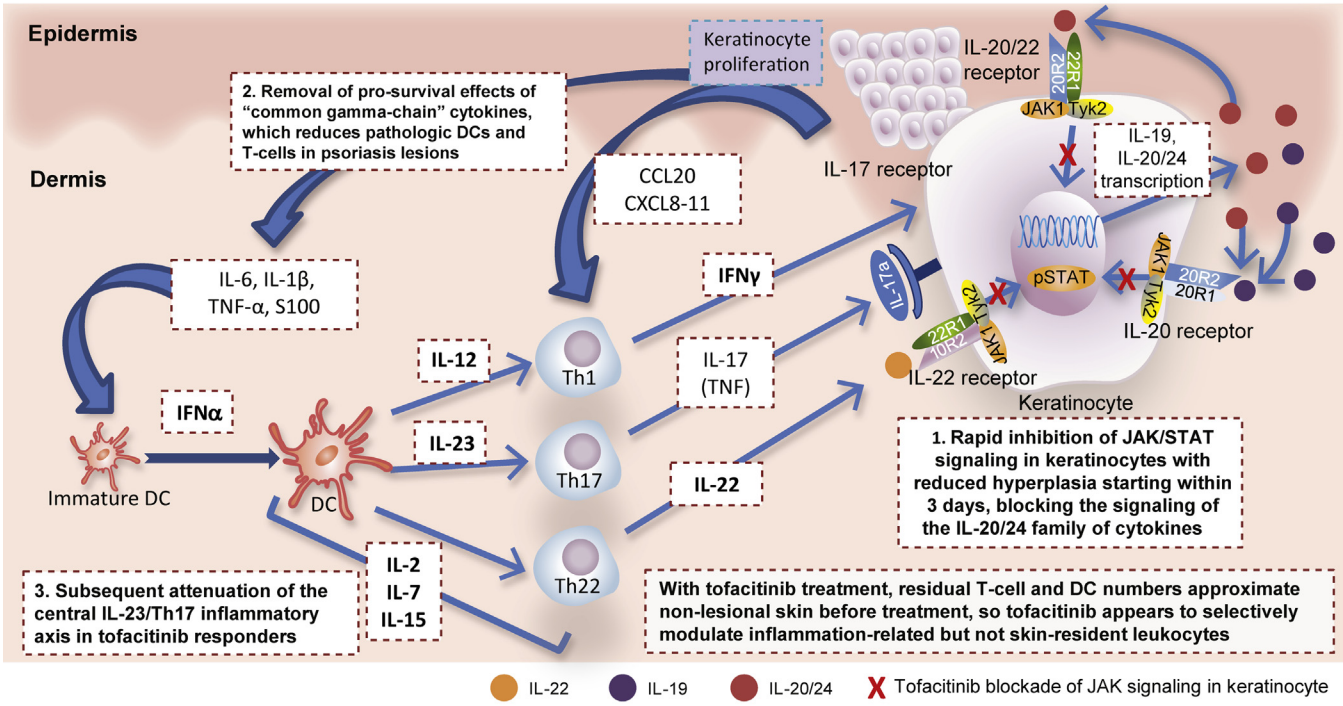



D, day; IL, interleukin; KRT16, keratin 16; LS, lesional skin; MAD3, meta-analysis of 3 psoriasis gene signatures; NL, nonlesional skin; PASI, Psoriasis Area and Severity Index; W, week.

**FIG 6. A,** Correlation between clinical (PASI score) and histologic (KRT16 level and epidermal thickness) outcomes and MAD3 psoriasis scores and IL-17 scores. **B,** Heat map showing mean expression profiles by histologic response to tofacitinib for genes in the psoriasis transcriptome that differ between responders and nonresponders at week 12 (FDR < 0.25; fold change > 2).

pSTAT1 and pSTAT3, markers of JAK signaling, were highly increased in keratinocytes in lesional skin and dramatically reduced after 1 day of tofacitinib treatment. This blockade of JAK signaling in keratinocytes led, both directly and indirectly, to reduced signaling of the IL-20/24 family of cytokines, including IL-19. JAK inhibition also reduced CCL20 production by keratinocytes. Consistent with this direct effect on keratinocytes, levels of inflammatory mediators produced by keratinocytes, including defensins (or AMPs) and IL-36, were reduced within the first 2 weeks of therapy. In patients with psoriasis, expression of cytokines and other inflammatory molecules by keratinocytes recruits additional $CD11c^+$ DCs and $CD3^+$ T cells to lesion sites, leading to perpetuation of the inflammatory response.[22] By inhibiting production of these molecules and inhibition of JAK-dependent cytokines, such as IL-7 and IL-15 (which promote survival of resident DCs and T cells[23]), tofacitinib treatment led to a reduction in pathologic DC and T-cell numbers in psoriatic lesions after 1 to 2 weeks. After a reduction in T-cell and DC numbers in lesional skin, expression of IL-17, IL-22, and IFN-γ was reduced by week 4. After 12 weeks of tofacitinib treatment, residual T-cell and DC numbers approximated those in pretreatment nonlesional skin. Therefore tofacitinib appeared to selectively modulate inflammation-related, but not skin-resident, leukocytes. In addition, the observed reduction in IL-17 and IFN-γ expression might also, in part, have resulted from blockade of the JAK signaling required for $T_H1$ and $T_H17$ cell differentiation, as seen in preclinical models.[7]

In assessing the mechanism of action of tofacitinib, it is important to consider the level of cytokine inhibition achievable at the clinical dose of 10 mg twice daily. In studies using cells of hematopoietic lineage, tofacitinib preferentially inhibited cytokines that signaled through JAK1, JAK3, or both, with less potency against JAK2- and tyrosine kinase 2–dependent cytokines (eg, IL-23).[6] Dosed at 10 mg twice daily, tofacitinib inhibited common γ-chain cytokines, such as IL-21, by approximately 70%; IFN-α by 80%; and IL-23 by 30% during the dosing period. At the minimum tofacitinib plasma concentration each day, the predicted inhibition was significantly less.[24,25] In studies using cultured keratinocytes stimulated with IL-22, the measured half-maximal inhibitory concentration predicted that IL-22 would be inhibited by an average of 30% through the day (data on file, Pfizer Inc), although more complete inhibition was observed in the biopsy specimens here. Although tofacitinib does not directly inhibit IL-17, it does inhibit $T_H17$ cell differentiation by inhibiting IL-21, IL-6, and, to a lesser extent, IL-23.[7] Therefore, at the clinical dose studied here, efficacy was derived from partial inhibition of multiple cytokines.




CCL20, chemokine (C-C motif) ligand 20; CXCL, chemokine (C-X-C motif) ligand; DC, dendritic cell; IFN, interferon; IL, interleukin; JAK, Janus kinase; pSTAT, phosphorylated signal transducer and activator of transcription; STAT, signal transducer and activator of transcription; Th, T-helper; TNF, tumor necrosis factor; TYK, tyrosine kinase.

**FIG 7.** Tofacitinib has a unique multitiered mechanism of action with an early direct effect on keratinocytes.

This study also evaluated the association between improvements in clinical disease severity and changes at the cellular and genomic levels in patients. We showed that tofacitinib reduced expression of psoriasis transcriptome genes, with changes occurring within 1 week and before reduction of IL-17 levels. In addition, the correlation between the MAD3 psoriasis transcriptome score and epidermal thickness was greater than the correlation between epidermal thickness and the IL-17 score, suggesting that additional pathways than IL-17 were targeted by tofacitinib that were important for early disease resolution.

A potential limitation of this study was the relatively small number of patients in the placebo group (n = 3). Variable changes were observed among those patients for clinical end points, which is likely related to natural fluctuations in psoriasis severity and PASI scores over time. Despite variable placebo group data, the reported correlation of clinical and histologic measures with MAD3 scores and IL-17 levels supports the conclusion that the observed molecular changes are associated with improvements in clinical and histologic outcomes.

In summary, these results demonstrate that partial inhibition of JAK signaling by tofacitinib results in a multitiered intervention in the cycle of psoriasis pathogenesis, with a direct effect on dysregulated keratinocytes, reduction in inflammatory infiltrate, and, ultimately, normalization of the IL-23/$T_H$17 axis.

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**Key messages**

- **Tofacitinib treatment leads to rapid attenuation of the increased JAK/STAT signaling seen in psoriatic keratinocytes.**
- **T-cell and DC infiltrates are increased in lesional skin. Tofacitinib reduced cell numbers to those seen in nonlesional skin, with a concurrent reduction in levels of cytokines that support the IL-23/$T_H$17 inflammatory pathway.**
- **Improvements in clinical and histologic features with tofacitinib are strongly associated with changes in expression of psoriasis-related genes and reduction in IL-17 expression.**

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